PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)* 16.06.2005

Applicant's or agent's file reference
21810 WO-HH

IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/003457	01.04.2004	04.04.2003

Applicant
ROCHE DIAGNOSTICS GMBH et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:

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Form PCT/IPEA/416 (January 2004)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 21810 WO-HH	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP2004/003457	International filing date *(day/month/year)* 01.04.2004	Priority date *(day/month/year)* 04.04.2003
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant ROCHE DIAGNOSTICS GMBH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04.11.2004	16.06.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Madlener, M Telephone No. +49 89 2399-7705

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP2004/003457

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-36 as originally filed

Claims, Numbers

1-17 received on 03.11.2004 with letter of 29.10.2004

Drawings, Sheets

1/15-15/15 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-14, 16
	No:	Claims	15, 17
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-17
Industrial applicability (IA)	Yes:	Claims	1-17
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item V.

The following documents are referred to in this communication:

D1: US 6,150,107 (November 21, 2000)
D2: US 6,177,247 (January 23, 2001)
D3: Ju J et al. (1995), Anal. Biochem., Vol. 231, pp. 131-140
D4: US 5,869,255 (Feb. 9, 1999)
D5: EP 0 640 828 (March 1, 1995)
D6: WO 97/46714 (December 11, 1997)
D7: US 6,197520 (March 6, 2001)
D8: Corbett Research: "Rotor-Gene 3000" (internet print-out)
D9: WO 98/49340 (November 5, 1998)
D10: US 6,369,893 (April 9, 2002)
D11: Vet JAM et al. (1999), Proc. Natl. Acad. Sci., Vol. 96, pp. 6394-6399

With regard to applicant's response to the written opinion, comprising an amended set of claims, it is noted that no amendment is apparent when comparing it with the previously filed set of claims discussed in the written opinion.

Applicant's arguments have been carefully considered. However, the authority entrusted with international preliminary examination (IPEA) cannot agree that the set of claims presently on file complies with the requirements of Article 33 (2) and (3) PCT.

NOVELTY:

1. It is acknowledged that **D1-D3** do not directly and inevitably at least 3 pairs of FRET hybridization probes suitable for multi-color real time PCR according to the present application. Hence, **claims 1-14** are considered to comply with the requirements of Article 33(2) PCT.

2.1 In response to the IPEA's previously raised argument, that D8 discloses a real-time PCR instrument according to claims 15 and 17 (cf. D8, whole document) and is thus considered to destroy the novelty of said claim pursuant to Article 33(2) PCT, applicant has argued that the real-time PCR instrument according to D8 has only 4

detection channels, whereas the real-time PCR instrument according to the present application comprises the "5-6 fluorescent entities" required by said claims, and that D8 does therefore not anticipate their subject-matter.

In view of the claim language "detector entities", the IPEA cannot agree with applicant's argumentation. Namely, D8 explicitly discloses "detection filters: 510 nm, 555 nm, 610 nm bandpass, 660 nm, 580nm, 610nm high-pass" (cf. D8, p. 8), i.e., "the standard four channels for multiplexing and an additional two filters for specialised applications" (cf. D8, p. 4). These four channels and two filters are considered to fall under the "5-6 detection entities" of claims 15 and 17. Given the fact that D8 further discloses that the real-time PCR instrument disclosed therein "can detect all available real-time chemistries including Sybr-Green, dual-labelled and MGB probes, FRET and Molecular Beacons" (cf. D8, p. 2) and comprises 4, i.e., at least 1 light source (cf. D8, p. 4) as well as heating and cooling means and multiple reaction vessels (D8, p. 4), the IPEA still considers **D8** to destroy the novelty of **claims 15 and 17** pursuant to Article 33(2) PCT.

2.2 Moreover, it is noted that **D10** would likewise appear to destroy the novelty of **claim 15** (Article 33(2) PCT). Namely, D10 discloses a real-time PCR thermo-cycling instrument comprising "at least four light sources .. and .. at least four [fluorescence] detectors, .. thus .. at least four separate optical channels" (D10, col. 4, ll. 50-65; col. 12; Fig. 5), and is hence considered to disclose an instrument as defined in claim 15 (cf., e.g., D10, abstract; Fig. 13; col. 3, l. 40 - col. 5, 39) comprising (at least) 5 fluorescent "detector entities".

3. Since none of the available prior art documents individually discloses a real-time PCR instrument comprising "5-6 fluorescent detector entities" in combination with "exactly one light source", **claim 16** is considered to comply with the requirements of Article 33(2) PCT.

INVENTIVE STEP:

1. With regard to inventive step, applicant has essentially argued that

   - prior to the present invention, no functional example has been disclosed,

characterized in that 4 different FRET pairs have successfully been used in a multiplex detection assay (all 4 different FRET pairs are present in one solution of at one spacial location);

- non of the cited references or any combination thereof disclose or suggest sets of FRET hybridization probes ... comprising two probe molecules, ... for multi-color real-time PCR applications or for a PCR thermocycler instrument suitable for using said set of FRET hybridization probes;

- any addition of an additional FRET probe to the composition or reaction mixtures and the corresponding additional detection channel necessary for the PCR instrument to perform a multi-color real-time PCR assay is a major challenge and clearly comprises an inventive effort; ... the interference between said FRET hybridization probes becomes the major obstacle.

2. With regard to this, the IPEA would like to note the following:

- The first of applicant's above-cited arguments would appear to relate rather to novelty than to inventive step.

- **D6** discloses sets of FRET hybridization probes comprising two probe molecules for real-time PCR applications or for a PCR thermocycler instrument suitable for using said set of FRET hybridization probes (cf., e.g., D6, p. 9, ll. 14-33; pp. 74-76), including multiplex analysis (cf., e.g., D6, para. bridging pp. 6-7). Upon hybridization of said two probes with the target sequence, the donor and acceptor fluorophores are within 25 nucleotides of one another (D6, p. 9, ll. 14-33). D6 explicitly teaches

> "*[w]hen multiplex analysis in one PCR reaction is desired, it is common practice to use different fluorescent labels with distinguishable emission spectra to identify the multiple products. The analysis is complicated by the limited number of fluorophores available and the overlapping emission spectra of those fluorophores that are available*" (D6, p. 76).

Thus, the problem to be solved by the present application may be formulated as

the provision of further compositions comprising pairs of FRET hybridization-probes suitable for performing multiplex real-time PCR, characterized in that the enable simultaneous detection of at least three, preferably four or even more reactions.

This problem is solved by **claim 1** of the present application. However, this solution is considered to lack inventive step for the following reasons.

The above-cited passage in D6 implies that the solution of this problem resides in the availability of a sufficient number of fluorophores having as little overlap in terms of emission spectra as possible. The Mathies et al. publications (**D1-D4**), which relate to novel energy transfer dyes characterized by "an unusually high sensitivity", teaches that the use of these dyes as donors "improves the performance and usefulness of ... sets of two or more lables of different emission spectra used in combination" (cf., e.g., D1, col. 2, ll. 10-28). More specifically, D1 teaches that, for multiplex applications, one or more, preferably one, donor fluorophore can be used (cf., e.g., D1, col. 3, l. - col. 4, l. 4), in combination different acceptor fluorophores (cf., e.g., D1, col. 4, ll. 37-42). D1 explicitly teaches acceptor fluorophores with emission maxima in the range of about 450 to about 1000 nm, preferably of about 500 to about 700 nm (cf., e.g., D1, col. 4, ll. 5-19) and that the emission maxima of the individual acceptor fluorophores should be separated by at least 10 nm, more preferably at least 15 nm, and most preferably at least 20 nm (cf., e.g., D1, col. 4, ll. 37-45).

aber nur Theorie ⇒ Umsetzung ist eine sehr schwierige

In view of this teaching, the skilled person confronted with the above-formulated problem and starting out from D6 would have applied the teaching of, e.g., D1. Merely by doing so, i.e., in the absence of inventive step, he/she would have arrived at the subject-matter of claim 1, which is hence considered to contravene Article 33(3) PCT.

3. With regard to the remaining claims, the IPEA remains with the inventive step objections raised already previously in the written opinion:

3.1 Given the fact that the simultaneous use of one donor and four acceptors (cf., e.g., **D1**, Ex. 1; Fig. 3-4) and of at least four FRET donor-acceptor-pairs was known prior

to the priority date of the present application (cf., e.g., **D1**, col. 3, l. 60 - col. 5, l. 15); that the prior art teaches how far the individual emission maxima of the acceptor fluorophores should suitably lie apart (cf., e.g., **D1**, col. 4, ll. 37-45); that there existed a wide variety of commercially available fluorophors prior to the efffective date of the present application (**D1**, col. 4, ll. 20-36); that, e.g,. **D3 and D4** both individually disclose the use of 4 FRET fluorescein donors (cf., e.g., D3, abstract; D4, Fig. 1), and that, e.g., **D7** explicitly discloses four donor-acceptor combinations with fluorescein as donor and acceptors according to claims 7 and 8 as acceptors as "acceptable fluorophore pairs" (D7, col. 5, ll. 47-52), the additional features of dependent **claims 2-9** would not appear suitable to establish the inventive step required by Article 33(3) PCT.

3.2 In view of the fact that the use of the compositions of claim 1 for PCR is considered obvious (cf. section 2., *supra*) and that real time PCR instruments suitable for the purpose defined in claim 10 formed part of the prior art (cf., e.g., **D5-D10** and the discussion of these and/or similar instruments on pages 2-4 of the present application), the "systems" according to **claims 10-11** and the methods according to **claims 13-14** are, for reasons analogous to those formulated above, considered to contravene Article 33(3) PCT. It is noted that the the authors of the present application acknowledge the suitability of these prior art devices for multiplex real-time PCR (cf. page 2, line 5 - page 4, line 16).

3.3 Given the fact that, e.g., the Roche Diagnostics LightCycler mentioned in the present application (cf., e.g., p. 2, l. 27 - p. 3, l. 8) contains one light source, the additional feature of **claims 12 and 16** would, again for reasons analogous to those set forth above, not appear suitable to establish the inventive step required by Article 33(3) PCT.

Enclosure to letter of March 17, 2005
International Patent Application No. PCT/EP2004/003457
Applicant: Roche Diagnostics GmbH
Applicant's Ref.: 21810 WO-HH

New Patent Claims

1. A composition or reaction mixture suitable for performing multi-color real time PCR comprising at least 3, preferably 4-5 most preferably exactly 4 pairs of FRET hybridization probes, each pair of hybridization probes consisting of a FRET donor probe carrying a FRET donor moiety and a FRET acceptor probe carrying a FRET acceptor moiety having an emission maximum between 550 and 710 nm.

2. A composition or reaction mixture according to claim 1, wherein at least 3, preferably at least 4 and most preferably exactly 4 FRET donor moieties are identical.

3. A composition or reaction mixture according to claim 1, wherein all FRET donor moieties are identical.

4. A composition or reaction mixture according to claim 1, wherein at least 3, preferably at least 4 and most preferably exactly 4 FRET donor moieties are Fluorescein.

5. A composition or reaction mixture according to claim 1, wherein all FRET donor moieties are Fluorescein.

6. A composition or reaction mixture according to claims 1-5, wherein at least one additional FRET donor moiety is selected from a group consisiting of Atto425 and WI343.

7. A composition or reaction mixture according to claims 1-6, wherein one FRET acceptor moiety is selected from a group consisting of LC-Red 705, Cy5.5, and JA286.

8. A composition or reaction mixture according to claims 1-7,

wherein at least one, two or three FRET acceptor moieties are selected from a group consisting of Cy5, LC-Red 640, and LC-Red 610

9. A composition or reaction mixture according to claims 1-8, wherein one FRET acceptor moiety is selected from a group consisting of Rh6G and TAMRA.

10. A system for performing multi-color real time PCR, comprising

- a real time PCR instrument, and
- a composition or reaction mixture according to claims 1-9.

11. A system according to claim 10, characterized in that said real time PCR instrument comprises

- at least 1 light source, preferably an LED
- at least 4 and preferably 5-6 fluorescent detector entities, each of said entities having central detection wavelengths which are distinct from each other by at least 25 and preferably at least 30 nm

characterized in that said detector entities are capable of

- simultaneously detecting maximum fluorescene emission of at least 3, preferably 4 and most preferably 5 differently labeled FRET Hybridization Probe pairs,
- simultaneously detecting maximum fluorescence emission of at least 2 differently labeled TaqMan hybridization probes, and
- detecting maximum fluorescence emission of SybrGreenI

- means for heating and cooling
- multiple reaction vessels for containing a reaction mixture.

12. A system according to claim 11, characterized in that said real time PCR instrument comprises exactly one light source.

13. A method for amplifying and detecting multiple target DNA sequences comprising

a) providing a composition or reaction mixture according to claims 1-9,

b) subjecting said reaction mixture to a thermocycling protocol such that amplification of said multiple target sequences can take place,

c) monitoring hybridization of each of said pairs of FRET hybridization probes at least once after a plurality of amplification cycles.

14. A method according to claim 13, wherein hybridization is monitored at least once in a temperature dependent manner.

15. A real time PCR instrument comprising

- at least 1 light source, preferably an LED
- 5-6 fluorescent detector entities, each of said entities having central detection wavelengths which are distinct from each other by at least 25 and preferably at least 30 nm,

characterized in that said detector entities are capable of

- simultaneously detecting maximum fluorescene emission of at least 3, preferably 4 and most preferably 5 differently labeled FRET Hybridization Probe pairs,
- simultaneously detecting maximum fluorescence emission of at least 2 differently labeled TaqMan hybridization probes, and
- detecting maximum fluorescence emission of SybrGreenI

- means for heating and cooling
- multiple reaction vessels for containing a reaction mixture.

16. A real time PCR instrument according to claim 15 comprising exactly one light source.

17. An instrument according to claim 15-16, characterized in that said central detection wavelengths are selected from a group of range of wavelengths, said group conisiting of 520-540 nm, 545-565 nm, 570-590 nm, 600-620 nm, 630-650 nm, 660-680 nm, and 700-720 nm.